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Changes in the structure of *Arabidopsis thaliana* roots induced during development of males of the plant parasitic nematode *Heterodera schachtii*

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Abstract

Plant parasitic nematodes of the genus *Heterodera* show a high degree of sexual dimorphism, which is reflected by different nutritional demands and differences in the structure of the induced specific syncytial feeding site in the plant. The determination of the sex of the nematode *Heterodera schachtii* and other related species was repeatedly reported to be dependent on trophic factors, which are provided by the induced syncytia. The structural differences of syncytia induced by *H. schachtii* in roots of *Arabidopsis thaliana* were analysed at the anatomical and ultrastructural level. Syncytia of males were induced in the root pericycle. The developing syncytium then expanded into procambial or cambial cells of the vascular cylinder. Differentiated vascular elements were not included. The expansion of the syncytium triggered the proliferation of cambial and peridermal tissues, in a manner similar to secondary growth, and the formation of additional xylem and phloem elements. In comparison to syncytia associated with females, syncytia associated with males were less hypertrophied and were composed of more cells. Distinct cell wall openings were mostly found between the few strongly hypertrophied syncytial elements at the actual feeding site in the pericycle. The ultrastructure was very similar to female-associated syncytia but showed conspicuous differences in the structure and localization of cell wall ingrowths. These ingrowths were rare and weakly developed and occurred not only at the interface with xylem elements but also at the internal and external walls of the syncytia. After feeding had ceased at the end of the third developmental stage the syncytia degenerated.

Abbreviations: J2 – second stage juvenile; J3 – third stage juvenile; J4 – fourth stage juvenile.

Introduction

Sedentary plant parasitic nematodes have evolved numerous specific adaptations to their habitats (Grundler and Wyss, 1995). One common feature of this group of nematodes is the induction of specific feeding structures in the host plants. An example of the extent to which the parasites have adapted is the mechanism of sex determination in cyst nematodes. Under favourable environmental conditions the majority of the juveniles develop to females that swell during development and acquire a lemon-like body shape, under adverse conditions the proportion of males increases. In the adult stage males become vermiform and mobile again. This phenomenon has been explained by two contrary concepts: either a particular sensitivity to environmental influences causes a selective mortality of females (Triantaphyllou, 1973), or the sex of juveniles is determined under the influence of environmental factors (Mugniery and Bossis, 1985; Grundler and Wyss, 1995). A number of studies strongly supports the latter concept of environmentally influenced, epigenetic sex determination. By a variation of nutritional factors or parasitism in plants of different host suitability it was possible to raise the proportion of one sex, whereas the mortality of the whole tested population remained stable (Betka et al.,

1991; Grundler et al., 1991; Mugniery and Bossis, 1985; Müller, 1985).

In ostensible contradiction to these findings is the fact that most cyst nematodes are amphimictic and thus the development of both sexes is essential. Therefore, an interaction between the nematodes and their hosts is required that on the one hand is able to influence sex determination but on the other hand secures the development of both sexes under any circumstances.

The syncytial feeding structure in the root is regarded as the main site of interaction between the plant and the developing nematode. Male and female juveniles have repeatedly been reported to be associated with syncytia of different sizes and volumes (Caswell-Chen and Thomason, 1993; Kämpfe, 1960; Kerstan, 1969, Müller et al., 1981). This morphological difference, however, has never been analysed in terms of its anatomical structure and location.

Arabidopsis thaliana is a useful model in plant pathology (Dangl, 1993) and is a good host of *H. schachtii* and other sedentary as well as migratory nematodes (Sijmons et al., 1991). The genetical and anatomical simplicity of this plant renders it a good model for studying interactions with plant parasitic nematodes (Wyss and Grundler, 1992). Grundler et al. (1994) and Golinowski et al. (1996) described the structure and development of the specific syncytial feeding sites induced in the roots by female nematodes. They showed clearly that the invading juveniles seek a procambial or cambial cell, which is selected as the initial syncytial cell. From this single cell the development of the syncytium originates.

We report here the first detailed analysis of the structure of syncytia of male juveniles and an assessment of the differences to those of female juveniles.

Materials and methods

Plant and nematode culture

Seeds of Arabidopsis thaliana ecotype Landsberg erecta (La-er) were surface-sterilized by shaking them for 2 min in 70% ethanol, 4 min in 5% calcium hypochlorite and subsequently rinsing them three times with sterile water. The seeds were then transferred into 9 cm plastic Petri dishes, containing 7 ml nutrient medium on the base of 0.7% Daichin agar (Brunschwig, NL) with a modified 0.2 diluted 'Knop' mineral composition with 0.1% Gamborg's B5 vitamin solution (Serva, GER) and 2% sucrose (Sijmons et al., 1991). The plants were kept at 25 °C in a light regime of 16 h throughout development.

After 10 days, batches of about 100 freshly hatched *Heterodera schachtii* second-stage juveniles (J2), obtained from sterile agar stock cultures (Grundler, 1989), were transferred to the *A. thaliana* plants in an aqueous suspension. After invasion of the juveniles, the plants were inspected daily under a dissecting microscope. Syncytia that were associated with a juvenile of which the sex could be clearly determined were sampled. The sex of the juveniles was determined on the base of the differentiation of the genital primordia as described by Grundler (1989).

In vivo *video light microscopy*

Plants were grown on a thin layer of the agar medium described above on a glass coverslip, inoculated and then transferred into an observation chamber (Wyss, 1992). Within the chambers, nematodes were able to develop to adult stages. Observations were made with a Reichert-Jung Polyvar light microscope. The video system consisted of a Hamamatsu C-2400 video camera and a Hamamatsu DVS-3000 contrast enhancement device. Recordings were made by a Panasonic AG-6720 video recorder on Super VHS tapes.

Analysis of fixed material

Samples were taken by dissecting segments of roots containing feeding sites and associated nematodes, according to developmental stages. In total, 57 specimens at the different developmental stages were analysed. The specimens were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) for 2 h, rinsed 4 times in cacodylate buffer and post-fixed in a solution of 2cacodylate buffer for 2 h at 4 °C. Then they were dehydrated in an increasing ethanol series, substituted by propylene oxide and infiltrated with Spurr's resin. The specimens were then transferred into flat moulds and the resin was polymerised at 65 °C for 8 h. Semi-thin (2 μm) and ultra-thin (60 nm) sections were cut with a Reichert (Leica) Ultracut E. Semi-thin sections were stained with 1% crystal violet. They were inspected with a Reichert-Jung Polyvar light microscope.

Ultra-thin sections were mounted on formvar coated 100 mesh grids and stained with a saturated methanolic solution of uranyl acetate followed by lead citrate. Examinations were made on a Siemens 101 transmission electron microscope at 80 kV.

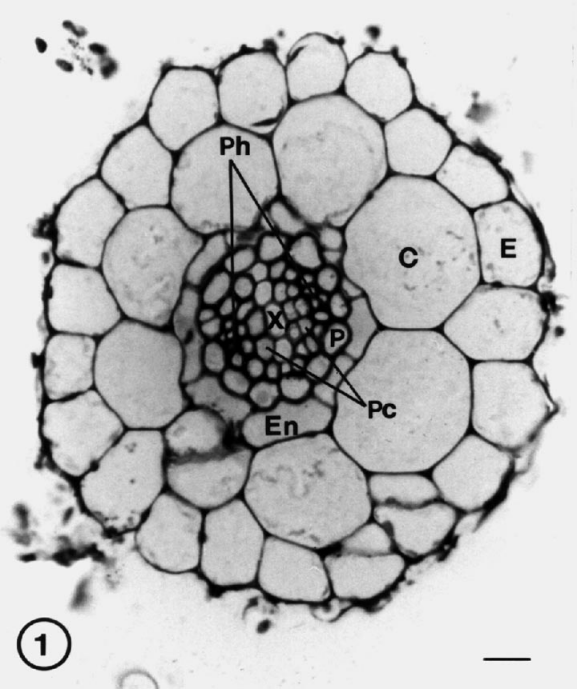
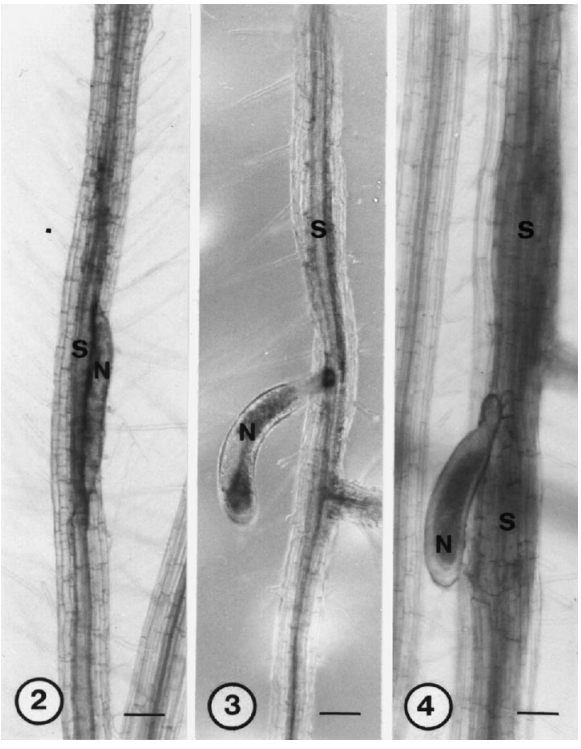


Figure 1. An uninfected lateral root of *A. thaliana* in the zone of elongation of a 2-week-old plant. C-cortex, E-epidermis, En-endodermis, P-pericycle, Pc-procambium, Ph-phloem, X-xylem. Bar 10 μm.



Figures 2–4. Morphology of the infection sites of *H. schachtii* in roots of *A. thaliana*. N-nematode; S-syncytium. Bar 100 μm. (2) J2, four days after infection. (3) Male J3, 8 days after infection. (4) Female J3, 8 days after infection.

Results

Anatomy of non-infected roots

The primary structure of *A. thaliana* roots (Figure 1) is composed of single cell layers of epidermis, cortex, endodermis and pericycle. The primary xylem bundles contain five to seven tracheary vessels. Two phloem bundles are positioned at both sides of the xylem axis. The space between the conductive bundles is filled with procambial cells. The diameter of the largest cortical cells is about 25 μm, while the central cylinder cells are about 6 μm in diameter. During secondary growth, the tissue forming the outer covering degenerates and is replaced by the periderm deriving from divisions of the pericycle. The procambial cells differentiate to a cambium from which a secondary conductive tissue develops. A detailed description of the developmental anatomy of the *Arabidopsis* root has been given by Dolan et al. (1993). In our description we follow the nomenclature proposed by these authors.

Nematode penetration and anatomy of infected roots

The J2 invaded along the entire length of the roots in regions of primary and secondary growth. For histological and ultrastructural analysis, mainly syncytia induced in the zone of elongation were selected. In this region the interpretation of sections was facilitated by the structural simplicity of the root.

During the first days after infection, the site of penetration was marked by only slight necroses of epidermal and cortical tissue. After establishment of the syncytia the juveniles grew rapidly and assumed a bottle-like shape. Within the thin transparent roots they could be localized easily (Figure 2). Eight days after invasion, after the moult to the J3 juvenile, the nematodes had emerged through the covering tissue. The male juveniles could now be discriminated easily from female juveniles by the shape of their developing gonads.

Typically, the syncytia of male juveniles were inconspicuous (Figure 3), whereas those of females were clearly visible as an extended, hypertrophied

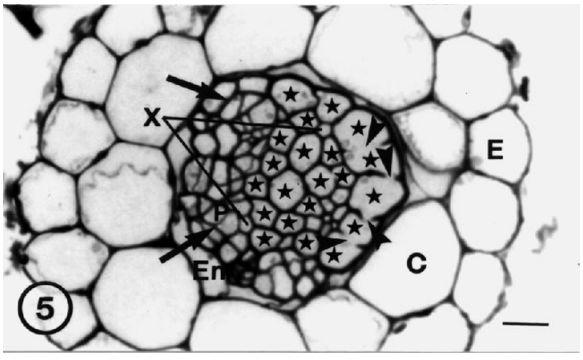


Figure 5. A syncytium (asterisks) of a 5-day-old (moulting) J2 male close to the head of nematode. The endodermis (En) is almost totally collapsed. Some pericycle cells are hypertrophied and incorporated into the syncytium while others underwent multiple divisions (arrows). Arrow heads-cell wall openings, C-cortex, E-epidermis, Pd-peridermis, X-xylem. Bar 10 μm.

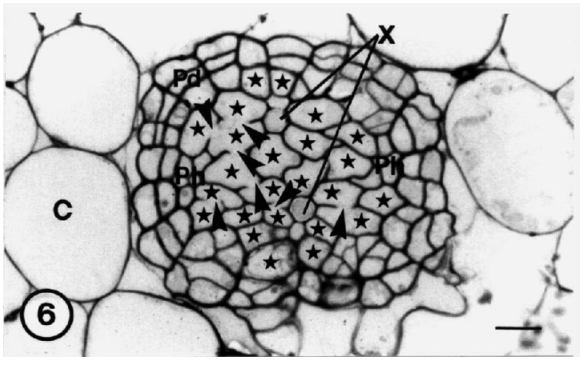


Figure 6. A syncytium (asterisks) of a 5-day-old J2 male about 300 μm away from the nematode's head. Note the openings (arrow heads) between the hypertrophied elements of the syncytium and cell divisions forming a peridermis (Pd). C-cortex, Ph-phloem, X-xylem. Bar 10 μm.

region in the vascular cylinder, causing distinct morphological alterations of the root (Figure 4).

Syncytia of J2 male juveniles

The earliest stage at which the juveniles' sex could be determined to be male was in the second developmental stage shortly before moulting to J3. A syncytium of such a specimen is shown in Figure 5. The volume of the central cylinder was only slightly enlarged. The endodermis was almost completely collapsed. Close to the nematode's head the syncytium consisted mainly of pericycle cells. They were fused by partial cell wall dissolution. At the opposite side of the vascular cylinder pericycle cells had undergone multiple irregular divisions.

At a distance of about 300 μm from the site of syncytium induction, the divisions of the pericycle became more regular, forming a peridermis similar to secondary growth (Figure 6). The degenerated endodermis and cortex have been replaced by peridermal cells. The syncytium in this region was located mainly in the centre of the vascular cylinder and primarily comprised procambial cells. Pericycle cells were not incorporated at regions remote from the induction site.

In cases where the nematode had invaded the vascular cylinder, the tissue in close vicinity to the nematode frequently became necrotic (Figure 7). These necroses not only concerned cells that had direct contact with the nematodes but also occurred in remote tissue. Necroses were interspersed with pericycle cells that had undergone multiple divisions, forming callus-like tissue or were strongly hypertrophied and incorporated into the syncytium. The proliferating pericycle cells later filled the space left by the degraded procambium. Xylem elements passing the necrotic area contained osmiophilic particles or were covered with a thin layer of osmiophilic material.

Syncytia of J3 male juveniles

Anatomy of syncytium induced at the primary stage of stage root growth

The expansion of the syncytia associated with males was completed at the end of the J3 as this is the last stage during which the juveniles feed; the J4 is a non-feeding stage. The anatomy of one of these syncytia is shown in Figures 8–22. The selected example did not show heavy necroses. The anatomy of developed syncytia induced in roots at the primary stage of growth always followed a general pattern. At the edge of the syncytium (Figure 8) facing toward the root tip, an undifferentiated central metaxylem precursor cell and a few procambial cells were slightly enlarged. Some other procambial cells had divided and the endodermis began to degenerate. Approaching the site of syncytium induction the first syncytial elements differentiated (Figure 9). These anteriorly situated elements usually originated from a central metaxylem precursor cell or its derivatives. The syncytial cells were distinctly hypertrophied. Further to the nematode, procambial cells were included into the syncytium and lateral roots were initiated from the pericycle (Figure 10). The syncytia never extended into these lateral roots. Closer to the nematode's head (Figures 11–13)

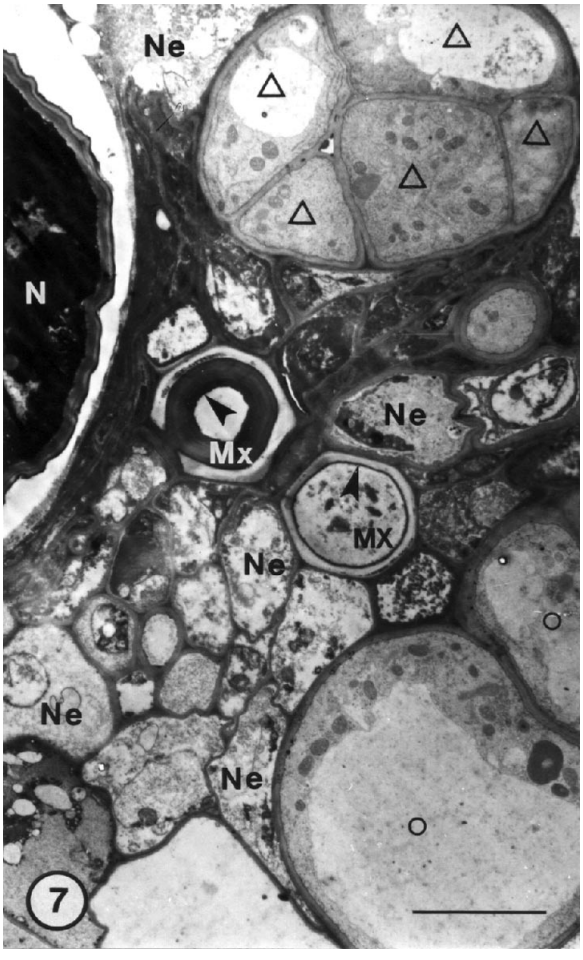


Figure 7. Ultrastructure of a vascular cylinder behind a syncytium associated to a J2 male (N). Almost all procambial and pericycle cells are necrotised (Ne). A few surviving pericycle cells had responded with division (triangles) or hypertrophy (circles). The surface of the inner wall of metaxylem elements (Mx) is lined with a thick layer of an osmiophilic substance (arrow head). Bar 4 μm.

the number of cells incorporated into in the syncytium increased. They were strongly hypertrophied and many of them originated from the pericycle. Cell wall openings were rare. At the site of induction, all cells of the central cylinder, except the fully differentiated xylem and sieve elements, were often incorporated into the syncytium (Figures 14–16). The pericycle syncytial elements at the site where the nematode fed (Figure 15) were strongly hypertrophied and connected by conspicuous and numerous cell wall openings, thus forming an area of confluent cytoplasm. Former procambial cells that were incorporated into syncytia were much less hypertrophied.

The changes caused by the development of the syncytium always caused some procambial or cambial cells to differentiate to additional secondary tracheary vessels (Figures 14–19). Additional xylem vessels differentiated often close to phloem bundles (Figure 26). The number of sieve elements was also increased. Up to fifteen sieve tubes were usually formed in direct contact with the syncytia (Figure 26), while in a similar uninfected root only 1 or 2 were present.

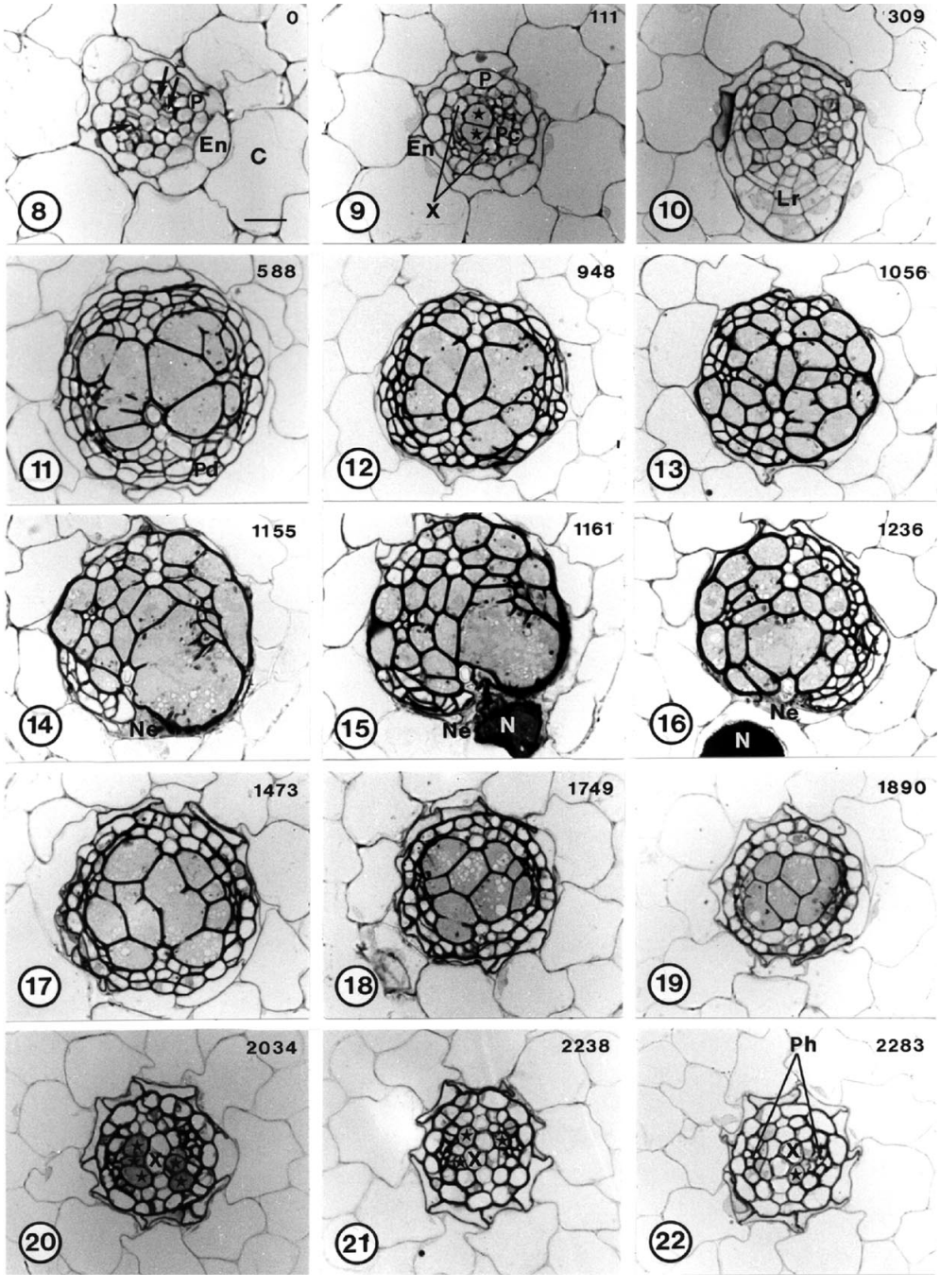
In the part of the syncytium facing towards the root base (Figures 19–22) the structural pattern described above was almost repeated. However, the distal syncytial elements were not created by the metaxylem precursors that were already fully differentiated. Here the procambial cells abutting primary xylem became the leading syncytial elements. The comparison of the central part of the syncytium (Figures 14–16) with both ends (Figures 9 and 22) showed a marked increase in cell division in tissues surrounding the syncytium.

Anatomy of syncytia induced at secondary stage of root growth

When an infection took place in the older part of the roots the syncytium in general had a similar structure to that described for syncytia induced in younger parts of roots. The most striking difference was the presence of a fully differentiated xylem (Figure 23). The syncytium then developed along both sides of the xylem bundle. According to the pattern of secondary growth, secondary xylem vessels differentiated from those cambial cells that had not been included into the syncytium. By divisions of the pericycle a thick layer of periderm had been formed. Close to the nematode head these divisions did not occur. Here the pericycle cells were included into the syncytium. They were strongly hypertrophied and connected by extensive cell wall dissolutions. However, only very few cell wall openings were present between cambial members of the syncytium.

Neoplasia

Sometimes strong hyperplastic responses of pericycle and cambial cells could be observed in relation to nematode infections. They were formed close to the syncytia at sites where substantial destruction had been caused by the activity of the invading nematode (Figure 24). The few surviving cells underwent multiple divisions and gave rise to a neoplastic callus-like tissue.



Figures 8–22. A series of cross sections of a syncytium (asterisks) associated with a J3 male. Numbers in the right upper corners indicate the distance from the first section (8) in μm. Beyond the root apical end of the syncytium only slight hypertrophy of some procambial cells and the precursor of the central metaxylem element occurs. Procambial cells show irregular divisions (arrows). Toward the syncytium the first hypertrophied syncytial elements (asterisks) appear and the endodermis (En) degenerates (9). The syncytium incorporates more and more cells of the vascular cylinder. Pericycle cells commence dividing and budding a lateral root (Lr) (10). Closer to the feeding site (11–17) of the nematode the syncytium integrates a greater part of the central cylinder. More and more pericycle elements are incorporated. The actual feeding site is strongly hypertrophied (15). Toward the root base (18–22) the syncytium consists mostly of former procambial cells thus reflecting the situation described in the acropetal part. However, in this older part of the root more cells are already differentiated to vascular elements that are never incorporated into a syncytium. Therefore, the distal syncytial elements are formed by procambial cells (asterisks) (20–22). C-cortex, Ne-necrosis, N-nematode, P-pericycle, Pc-procambium, Pd-Peridermis, Ph-phloem, X-xylem. Bar 10 μm.

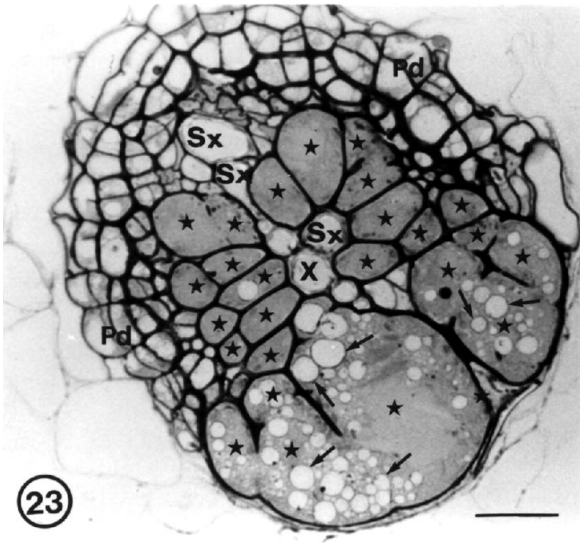


Figure 23. A syncytium (asterisks) of a male J3 during moulting to J4 induced in roots at an early stage of secondary growth. The pericycle has developed into periderm (Pd) consisting of several cell layers. The row of primary xylem (X) had already been differentiated before syncytium induction. Only undifferentiated cambial cell were integrated into the syncytium while elements of secondary xylem (Sx) differentiated from cells not integrated in the syncytium. The syncytium contains many vesicles (arrows) indicating the approaching degeneration. Bar 10 μm.

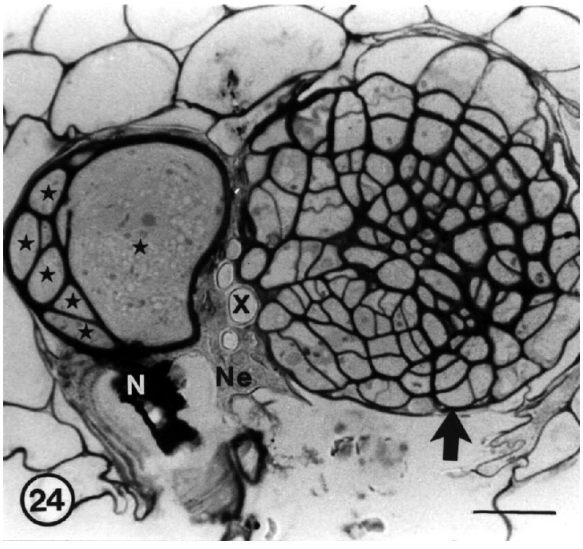


Figure 24. Neoplastic gall-like structure in infected root. Due to the migratory activity of the invading nematode (N) most cells of the vascular cylinder had been destroyed. Necroses (Ne) are visible in the area around the nematode. On the one side of the row of primary xylem (X) the syncytium (asterisks) developed, while on the other side multiple divisions led to the formation of a gall-like structure (arrow). Bar 10 μm.

Sometimes some cells of this tissue were incorporated into expanding syncytia.

Ultrastructure of the syncytia

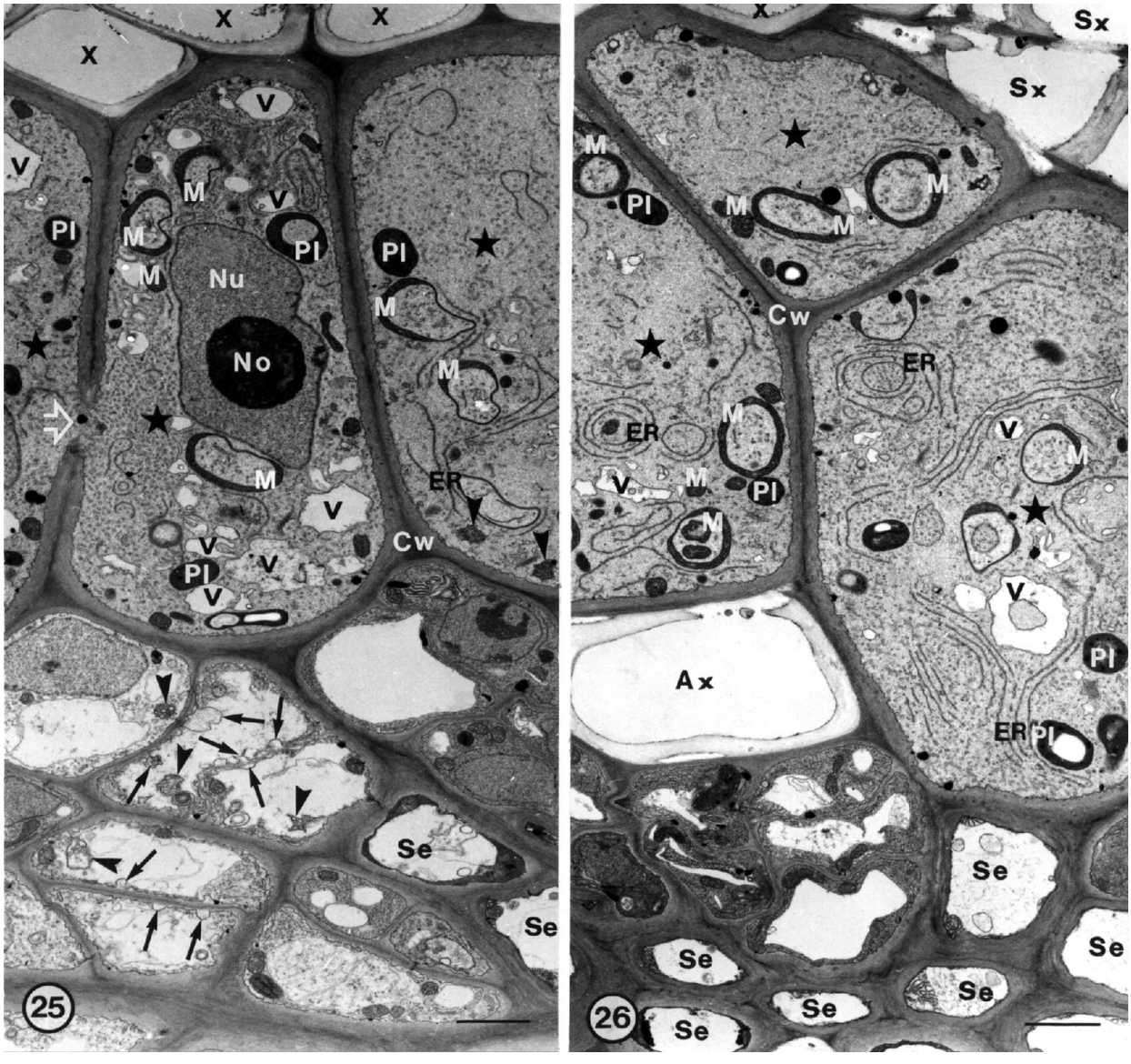
The protoplasts of syncytia were always highly condensed (Figures 25 and 26) and the plasmalemma was on close apposition to the cell walls but disappeared at cell wall openings. One of the most obvious changes, as already described by Golinowski et al. (1996), was the re-organisation of the vacuole. Instead of central vacuoles a system of small cytoplasmic vacuoles was established. These vacuoles had different shapes, sizes and contents (Figures 25–26). The nuclei of syncytia (Figure 25) were enlarged, lobed and possessed well preserved, darkly stained nucleoli.

Depending on the direction of sectioning, the mitochondria had round shapes or were elongated. They appeared normal with well developed systems of cristae (Figures 25 and 26). Plastids had weakly developed lamellar systems and they usually contained no starch grains. Only in distal syncytial cells the starch grains were commonly present in the plastids. All plastids contained few osmiophilic plastoglobules. Both plastids and mitochondria often acquired cup-like shapes and enclosed cytoplasm with other organelles inside (Figures 25 and 26).

Endoplasmic reticulum (ER) proliferated and created a complex membraneous system. The ER was often arranged in parallel layers or concentric swirls that sometimes enclosed groups of other organelles (Figures 25 and 26). The ER system was present in the entire syncytium and linked together through the cell wall openings. Occasionally groups of ribosomes were observed to be anchored on the membranes but usually they were detached from ER membranes and dispersed over the cytoplasm singly or as polysomes (Figure 29).

Ultrastructure of syncytial cell walls

The syncytial cell walls displayed drastic modifications (Figures 25–29). They were generally thickened and openings were formed by partial dissolution of wall material at certain sites. However, these openings were rare compared to syncytia associated with female nematodes (Golinowski et al., 1996). A typical feature of the syncytia was the development of cell wall ingrowths which appeared first at the beginning of the J3 stage. They formed at sites abutting xylem



Figures 25–26. Ultrastructure of a syncytium (asterisks) of a J3 male and neighbouring cells. The condensed syncytial cytoplasm is rich in smooth endoplasmic reticulum (ER), plastids (Pl) and mitochondria (M). ER is frequently arranged in parallel rows or concentric swirls (26). Small vacuoles (V) have replaced the central vacuole. New thick-walled sieve tubes (Se) and additional xylem elements (Ax) have differentiated in the vicinity of the syncytium. The hypertrophied and lobed nucleus (Nu) with a darkly stained nucleolus (No) is visible (25). Paramural bodies (arrows) and multivesicular bodies (arrow heads) are formed abundantly in neighbouring cells. Cell wall openings are indicated by open arrows. CW-cell wall. Bars 3 μm.

tracheary elements (Figure 27). Sometimes they were also created on inner and outer syncytial walls (Figures 28 and 29). The shape of the ingrowths differed considerably from that of ingrowths in syncytia associated with females. They were weakly developed and tended to fuse and to form an irregular mass of wall material.

Syncytia of J4 male juveniles

At the end of the J3 stage the male juveniles ceased feeding. The first symptoms of degradation of the syncytia appeared gradually. During the moult to J4 the number and size of vacuoles occurring in the syncytial cytoplasm increased (Figure 30). The cyto-

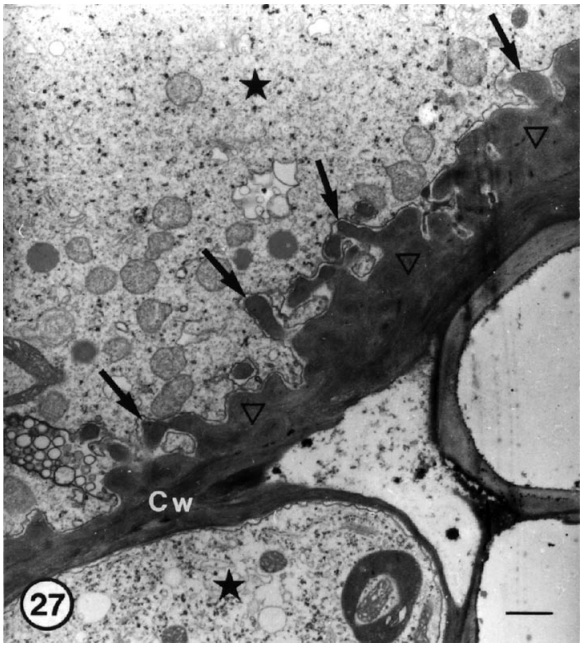


Figure 27. Cell wall ingrowths (arrows) in the syncytium (asterisks) of a J3 male located at the interface to xylem vessels (X). The ingrowths are partially fused to form a thick layer (triangles) on the cell wall (Cw). Bar 1 μm.

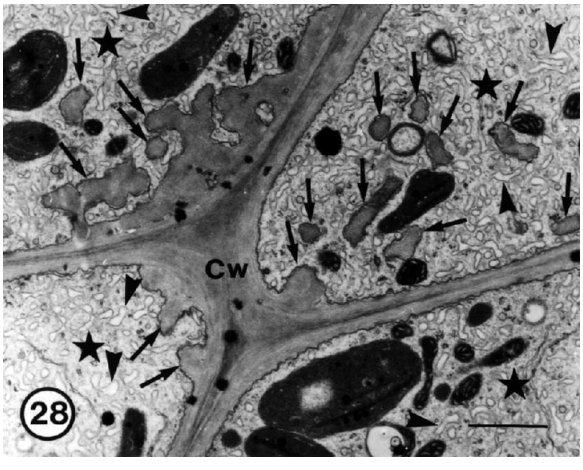


Figure 28. Cell wall ingrowths (arrows) in the syncytium (asterisks) of a J3 male located at internal syncytial walls (Cw). Arrow heads indicate ER tubules. Bar 1 μm.

plasm became progressively electron translucent and the nucleoplasm stained only faintly, while the nucleoli retained their dark appearance. The entire ER system gradually disappeared, although mitochondria and plastids remained preserved. These degradations were restricted to the syncytial elements. Other vascular cylinder cells did not degenerate, although xylem elements were often found to be filled with osmiophilic material.

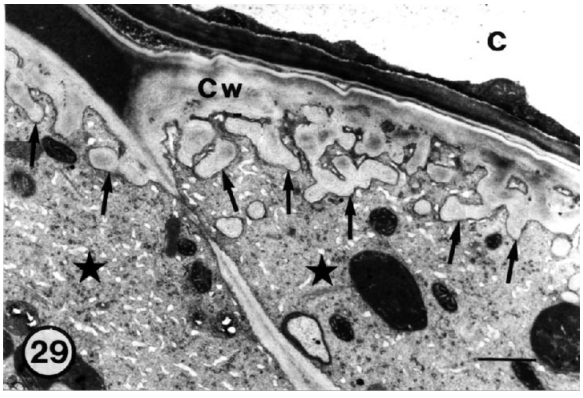


Figure 29. Cell wall ingrowths (arrows) in the syncytium (asterisks) of a J3 male located at the outer syncytial cell wall (Cw) facing the cortex (C). Bar 1 μm.

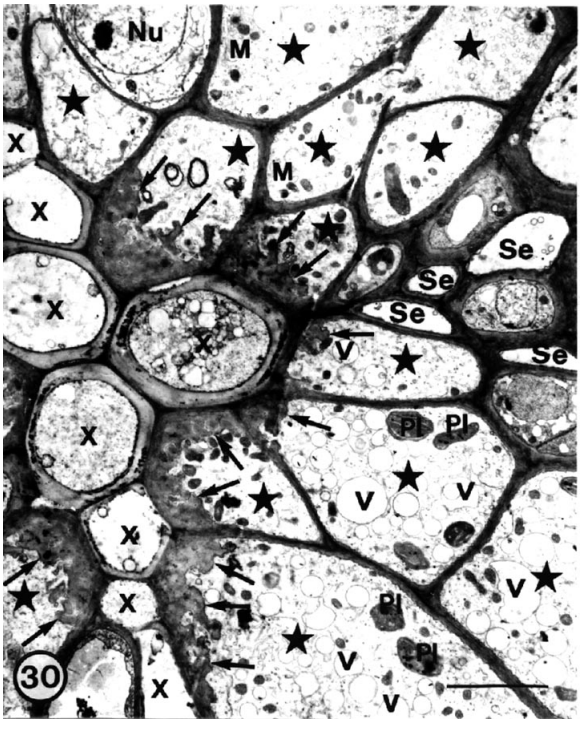


Figure 30. Degenerating syncytium (asterisks) of a J4 male at some distance to the actual feeding site. The cytoplasm is filled with vesicles (V) and the nucleus (Nu) is electron translucent. Cell wall ingrowths (arrows) create a compact layer similar to cell wall thickenings. Xylem vessels (X) contain a fibrillar substance and vesicular material. Mitochondria (M) and plastids (Pl) are still well preserved. Se-sieve elements. Bar 4 μm.

Discussion

As indicated by its genus name, males and females of *H. schachtii* exhibit a strong sexual dimorphism. The obvious morphological differences are related to different physiological demands. Female nematodes were shown to consume twenty nine times more food than males during their entire development Müller et al., 1981). In sugar beet they occupied minimum root volumes 3.7 times greater than minimum root volumes of males (Caswell-Chen and Thomason, 1993). Our investigations were performed in order to characterize the differences between syncytia of males and females at the anatomical and ultrastructural level.

Once having established an initial syncytial cell (ISC), cyst nematodes are no longer able to change their feeding site (Wyss and Zunke, 1986). The induction site of syncytia therefore can be deduced with some restrictions from the location of the head of nematodes that are already in an advanced developmental stage. Golinowski et al. (1996) showed that syncytia of females were always induced in procambial or cambial cells depending on the developmental stage of the root. In contrast, we observed males to select the pericycle for syncytium induction. It is still not known whether cells that are selected as ISCs have to meet specific demands. Reporter-gene studies revealed that promoters of cell-cycle regulating genes are activated shortly after syncytium induction (Niebel et al., 1994). It is possible that selected cells have to be in a certain stage of the cell cycle or the cell differentiation to be able to respond by forming a syncytium. It is also known from *A. thaliana* and other plants that the levels of ploidy are different in different cell types (Melaragno et al., 1993). Endoreduplication was found to be typical for cells in early stages of xylem differentiation (Innocenti and Avanzi, 1971; Lai and Srivastava, 1976). Whether these or other factors are decisive is not known. It can only be stated that all cell types observed to serve as ISC, procambium, cambium and pericycle, have a high meristematic potential and a low degree of differentiation.

ISCs associated with male juveniles were found in the outer region of the vascular cylinder where the nutrient supply may be less than in more centrally located cells. Interestingly, the syncytia induced in the outer region of the central cylinder tended to expand into the centre by incorporating of procambial or cambial cells. The distal, most recently included syncytial elements in advanced stages of syncytium development were only procambial or cambial cells next to xylem elements.

One of the most striking differences between syncytia of males and females was found to be the structural composition. Whereas syncytia of female juveniles were composed of a relatively low number of strongly hypertrophied cells (Golinowski et al., 1996), those of male juveniles sometimes included almost all the cells of the vascular cylinder which, however, were only slightly hypertrophied. The ISC and some surrounding cells, which were incorporated into the syncytia early, showed the highest degree of hypertrophy. These cells also showed the most pronounced cell wall openings. The extent of opening seems to be positively correlated with the extent of hypertrophy. Thus, cell wall openings were found only rarely between syncytial elements that were only slightly hypertrophied. Again, in syncytia associated with females cell wall openings were very conspicuous (Golinowski et al., 1996). Injection of fluorescent markers into syncytia of both males and females indicated that the syncytial continuity of the cytoplasm and the symplastic isolation of the syncytia occurred in both types of feeding structures (Böckenhoff and Grundler, 1994).

Most ultrastructural features of the syncytia of male juveniles resembled that described for syncytia associated with females in *A. thaliana* (Golinowski et al., 1996) and other compatible plant-cyst nematode interactions (Bleve-Zacheo and Zacheo, 1987; Endo, 1986; Golinowski and Magnusson, 1991; Magnusson and Golinowski, 1991; Wyss et al. 1984). A conspicuous difference was the appearance and location of cell wall ingrowths. These structures were described many times as specific features of transfer cells and feeding structures induced by sedentary nematodes (e.g. Jones and Northcote, 1972; Wyss et al., 1984). In syncytia associated with females they occurred from the J3 stage onwards as well developed protuberances on cell walls abutting xylem elements. In the case of male-associated syncytia the cell wall ingrowths developed as weak invaginations of cell walls that tended to fuse to an uneven wall deposition. They were not only found at the interface with xylem elements but also at internal and outer syncytial walls. These observations are difficult to interpret. The precise function of the ingrowths is still not known. Usually they are regarded as improving short distance transport. However, speculations on the substances transported vary from minerals and amino acids (Atkinson, 1995) to just water (Böckenhoff and Grundler, 1994). The fact that they appeared in male-associated syncytia at the out-

er syncytial wall could be an indication of a shortage of certain substances. However, based on the ultrastructural observations, a different metabolic activity between syncytia associated to males or females could not be observed.

The processes occurring in syncytia and the entire nematode development were well co-ordinated. In general, it is difficult to discriminate between trigger and response: are the structural differences due to the development of male or female juveniles or do they cause male or female development? Supposing plant factors were able to influence sex determination, the signals which may lead to male or female formation still have to be determined. As first features of sexual dimorphism already occur at the end of the J2 stage (Grundler, 1989; Wyss, 1992), the triggering signal must be perceived before this stage either in the form of trophic stress, certain cellular responses, a particular factor or the combination of several factors. At the structural, biochemical or molecular level this problem can only be solved if samples can be taken from plants that can be manipulated in a way that enables the sex of the invading juveniles to be predicted (Grundler, 1989). Resistant plants that are known to allow only male development (Golinowski and Magnusson, 1991; Grundler, 1989; Müller, 1985) have a genetic background different to susceptible plants and, therefore, may be not suitable for such experiments.

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